

Applicant : Shih-Jen Liu, et al
Serial No. : 10/072,185
Filed : February 8, 2002
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Attorney's Docket No.: 13886-002001 / 01P0325

Replace the paragraph beginning at page 5, line 8, with the following rewritten paragraph:

--Hsp70 gene was amplified from human hepatocellular carcinoma HepG2 cDNA with a gene-specific forward primer: 5'-cgcgatccATGGCCAAAGCCGCGGC-3' (SEQ ID NO:1), and a gene-specific reverse primer: 5'-cgcgatccCTAATCTACCTCCTCAATGG-3' (SEQ ID NO:2). The 1.92 kb Hsp DNA fragment was cleaved with *Bam*HI and ligated with a *Bam*HI-cleaved pRSETA vector. After transformation, plasmids were prepared from an overnight culture of transformed *E. coli* clones, and further analyzed by restriction enzyme digestion and sequencing. The recombinant plasmid containing Hsp70 gene was named as pRSETA/Hsp70.--

Replace the paragraph beginning at page 5, line 16, with the following rewritten paragraph:

--Hsp C-terminal DNA fragment was amplified from pRSETA/Hsp70 with an HspC'-specific forward primer: 5'-gggaattcGCGATGCCAACGGCATCCTGAAC-3' (SEQ ID NO:3) and an HspC'-specific reverse primer: 5'-ggaaattCTAATCTACCTCCTCAATGGTG-3' (SEQ ID NO:4). The 0.5 kb HspC' DNA fragment was cleaved with *Apo*I and ligated with an *Eco*RI-cleaved pRSET vector. After transformation, plasmids were prepared from an overnight culture of transformed *E. coli* clones, and further analyzed by restriction enzyme digestion and sequencing. The recombinant plasmid containing Hsp C-terminal DNA fragment was named as pRSET/HspC', which served as a backbone for construction of tumor antigen-HspC' expression plasmids.--

Replace the paragraph beginning at page 5, line 26, with the following rewritten paragraph:

--HepG2 cells were homogenized in RNazolTMB solution, and total RNA was prepared according to the protocol provided with the kit. The cDNA was synthesized by SuperScriptTM II Reverse Transcriptase (GIBCO BRL) with an oligo-d(T)₁₂₋₁₈ primer. AFP gene was amplified from HepG2 cDNA with a gene-specific forward primer: 5'-gcggatccACACTGCATAGAAATGAATATG-3' (SEQ ID NO:5), and a gene-specific reverse primer: 5'-gcggatccAACTCCCAAAG

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CAGCACGAG-3' (SEQ ID NO:6). The 1.77 kb AFP DNA fragment was cleaved with *Bam*HI and ligated with a *Bam*HI-cleaved pcDNA3 vector. After transformation, plasmids were prepared from an overnight culture of transformed *E. coli* clones, and further analyzed by restriction enzyme digestion and sequencing. The recombinant plasmid containing AFP gene was named as pcDNA3/AFP.--

Replace the paragraph beginning at page 6, line 17, with the following rewritten paragraph:

--Total RNA was prepared from LNCaP cells with RNazolTMB (Tel-Test). LNCaP cDNA was synthesized by SuperScriptTM II Reverse Transcriptase (GIBCO BRL) with an oligo-d(T)₁₂₋₁₈ primer. PSA gene was amplified from LNCaP cDNA with a gene-specific forward primer: 5'-ATTGTGGGAGGCTGGGAGTG-3' (SEQ ID NO:7) and a gene-specific reverse primer: 5'-GGGGTTGGCCACGATGGTG-3' (SEQ ID NO:8). The PCR reaction was performed by DyNAzymeTM (FINNZYMES), and the 0.8 kb DNA fragment from PCR reaction was ligated to a pCRII vector (INVITROGEN) directly. After transformation, plasmids were prepared from an overnight culture of transformed *E. coli* clones, and further analyzed by restriction enzyme digestion and sequencing. The recombinant plasmid containing a sequence encoding the mature PSA was named as pCRII/mPSA.--